wax using a Leica ASP or Pelorus system with >1 hour formalin fixation. Sections of these were then cut at 10 microns and four sections pooled for DNA extraction, performed using a Ambion Recoverall™ total nucleic acid isolation kit optimised for FFPE samples. Quantitiative PCR detection of mutations present was performed using the ARMS Scorpions kit (DxS/Qiagen, Manchester, UK) according to the manufacturer's instructions with an AB7500 PCR machine (Applied Biosystems Inc, Foster City, CA).
Results: Sufficient DNA recovery was obtained using both processing methods, despite the higher temperature and xylene free processing used by the Pelorus, though slightly higher recovery was observed using the ASP processor. Histology of the cell line pellets is feasible and routinely performed for each pellet, and the results can be rapidly compared with response to drugs or candidate molecules in vitro.
Conclusion: Cell lines containing specific integrated mutations provide an ideal alternative to tumour samples for target validation and drug discovery, to which one can also now add companion diagnostic design, initial validation, and quality assurance.

646 POSTER

Identification of biomarkers associated with tumor progression using laser microdissected tissues from colon adenoma and cancer

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Background: Colorectal (CRC) malignancies rank worldwide at third place for tumor diseases and account for an annual mortality rate of 492 000 cases. Although several molecular events are known to be involved in the transition from normal tissue to adenoma and finally to undifferentiated carcinoma it remains a challenge to discover new and more reliable biomarkers for diagnosis, prognosis and prediction of outcome. Towards this end a study was designed to identify potential biomarkers which are associated with the molecular events leading from epithelial adenoma to the early stages of carcinoma.
Material and Methods: A new biomarker discovery strategy was developed to combine the cell specificity and the selectivity of laser capture microdissection (LCM) with the resolution power and sensitivity of liquid-chromatography (LC)-matrix-assisted-laser-desorption/ionization mass spectrometry (LC-MALDI-MS). We carefully selected a group of closely matched patients (n = 10 for each group) afflicted with epithelial adenoma (high dysplasia) or early stages of carcinoma (stage I) and used the derived normal as well as the matched tumor tissue samples to reveal protein expression differences. According to this LC-MALDI-MS strategy microdissected cells were lysed and extracted proteins were digested with trypsin. Obtained peptides were separated by capillary reversed phase HPLC (Agilent). The resulting LC-fractions (300) were spotted on prespotted AnchorChip targets (PAC, Bruker) and tryptic fragments subsequently detected by reflector MALDI-MS (ultraflex III,Bruker) measurements. Differential peptide analysis was performed to discover robust and significant expression differences between patient groups. Therefore, only m/z ions displaying a minimum twofold difference and a p-value of 0.01 between groups were considered for further analysis. The selected peptides were subsequently fragmented by MS/MS experiments to reveal their primary sequence and protein identity.
Results: Up to 7000 ion signals ranging from m/z 800 to 4000 were generated and used for statistical analysis. Our targeted biomarker discovery approach resulted in the identification of more than 30 biomarker candidates.
Conclusion: The identified biomarker candidates are involved in diverse cellular functions and can now probably be linked to early or late events of tumor progression. Currently these biomarkers are being validated using antibody based assays to further analyze their potential as markers in a clinical setting.

647 POSTER

Detection of miR-302, an ES-specific microRNA, in cancer cell lines and tissues

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Background: The miR302–367 is a cluster of microRNAs that are exclusively expressed at high levels in embryonic stem (ES) cells. Indeed, miR-302–367 cluster may play an essential role in maintaining hESC pluripotency and self-renewal. In addition, promoter of miR302–367 is transcriptionally regulated by the ES cell master regulators, e.g. Oct3/4, Sox2, and Nanog (OSN). Previous studies indicated that expression of OSN could be detected in tumor samples. Therefore, expression of miR302s as a downstream component of OSN is also conceivable in the cancer cells and has the merit of being studied as a potent tumor marker.
Material and Methods: Generally, amplification and detection of microRNAs by PCR is not straightforward, due to their short lengths (20−24nt). In addition, high similarity of miR302-family members, make it difficult to specifically detect individual members. In the current study, a stretch of A-nucleotides were added to the 3′-end of the extracted RNAs by using poly-A polymerase. cDNA was then synthesized using an oligo-dT primer that was anchored to a tag sequence on its 5′-end. The tag could be used as a reverse primer in the subsequent stages. Additionally, the forward primer was selected such that it could specifically amplify miR302b.
Results: Specificity of the PCR was examined using a vector containing miR-302a, miR-302c, and miR-302d but not miR-302b. Based on our data, the miR302b-PCR system was specific, at least in the presence of 6×10^6 copies of the vector in 45 cycles. Consequently, the system was evaluated in different tumor samples and cell lines. Interestingly, the expression of miR302b was detected in some brain and bladder tumor cell lines in addition to tumor samples.
Conclusion: Recently, we have shown the functionality of miR302–367 promoter in a rare sub-population of brain tumor cell lines. The results of the current study also demonstrated a low expression level of miR-302 in cancer cells. These results may indicate that upstream regulators of miR-302, namely OSN, are expressed in a rare sub-population of cancer cells. In other words, an ES-like expression pattern and stemness properties may exist in this sub-population of putative cancer stem cells.

648 POSTER

Ex vivo assay to monitor response to chemotherapeutic agents in plucked human hairs

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Background: Plucked hair is a valuable surrogate biomarker tissue to monitor pharmacodynamic (PD) responses. Hair collection is also minimally invasive, simple and permits frequent sampling. We have previously developed immunohistochemistry (IHC) labeling protocols for plucked human scalp hair, enabling target response to treatment to be monitored directly. We now present an assay for the *ex vivo* maintenance, treatment and labeling of human hair. This provides effective proof of concept data for the detection in hair of a target protein response, before progressing to a clinical trial.
Methods: Donor hairs were plucked and immediately transferred to a maintenance medium containing vehicle or 200 nM Tarceva for 2 or 10 mins before being harvested and fixed (5 hairs per donor, per treatment) along with freshly plucked untreated hairs (5 per donor). Hairs were then embedded in wax, sectioned and IHC labeled for phospho-ERK1/2 (p-ERK1/2) using methods previously developed. The amount of p-ERK1/2 present was quantified using the Ariol® slide scanning system.
Results: In fresh hairs the p-ERK1/2 was restricted to distinct bands across the outer root sheath (ORS) of the hair. In control hairs maintained *ex vivo*, there was some diffusion of the banded labeling after 2 mins and complete diffusion after 10 mins, with labeling then presenting throughout the ORS. Labeling in hairs treated for 2 mins with Tarceva was similar to that of hairs treated with vehicle for 2 mins, whilst labeling was greatly reduced in hairs treated for 10 mins with Tarceva compared to 10 min vehicle treated hairs. Labeling was quantified on the Ariol® scanning system and after 10 min treatment with Tarceva a significant 73%, 59% and 61% decrease in donor 1, 2 and 3, respectively, was observed compared to 10 min vehicle treated hairs (Students *t*-test: donor 1 p = 0.0002, donor 2 p = 0.01, donor 3 p = 0.007).
Conclusion: This preliminary data indicates good reproducibility in the *ex vivo* hair assay and a significant reduction in p-ERK1/2 levels following 10 min Tarceva treatment. The *ex vivo* hair assay provides valuable proof of concept biomarker data, prior to collection of hairs within a clinical study, confirming whether the hairs are suitable surrogates for the target protein of interest.

649 POSTER

Epidermal growth factor receptor (EGFR) gene amplification is not the cause of protein overexpression in penile carcinoma

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Background: Squamous cell carcinoma of the penis affects mainly people with poor hygiene habits in undeveloped countries. Epidermal growth factor receptor (EGFR) is a well characterized tyrosine-kinase receptor that has

its expression levels increased in diverse tumors, especially in carcinomas. Based on that, this study has aimed at evaluating the gene alterations associated with high protein expression levels.
Material and Methods: Immunohistochemistry (IHC) against EGFR was performed in 195 penile carcinoma samples selected from the files of AC Camargo Hospital, Brazil. Cases showing strong and complete membrane staining in more than 10% of the tumor cells were considered positive and were submitted to dual-color fluorescence *in situ* hybridization (FISH). Reactions were carried out using fluorescenet-labeled probes for EGFR locus and chromosome 7 centromere (Zytovision™) in samples over-expressing EGFR, previously selected by immunohistochemistry. Cases showing two signals of each probe were considered non-altered, those showing more than two signals of each probe were considered polissomyc and those showing more EGFR signals compared to centromere signals were considered amplified.
Results: In this series, 67 (49,7%) penile carcinoma samples overexpressed EGFR by IHC and were selected for FISH. Protein overexpression was associated with greater risk of recurrence in univariate analysis (p = 0,031). Regarding FISH, 31 cases (46%) were uninterpretable and, out of 36 valid cases, 22 (61,1%) were non-altered cases, 12 (33,3%) were polissomyc of cromossome 7 and 2 (5,6%) cases presented EGFR amplification.
Conclusions: The high number of uninterpretable cases in FISH seems to be related to technical artifacts due to the high quantity of cytokeratin which may block probe penetration in cytoplasm and nuclei of these tumor cells. Although EGFR overexpression seems to be associated with worse prognosis, neither gene copy number nor polysomy of chromosome 7 is the main cause of this abnormality in penile tumors. Further studies concerning mutational analysis and clinical data are needed and might be useful for identifying patients who may benefit from EGFR-target therapy.

650 POSTER

Multiparameter PET imaging for assessing risk/outcome in sarcoma

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Background: PET molecular imaging with biologically specific agents has the potential to assess phenotypic expression for multiple tumor pathways non-invasively and serially during treatment. To demonstrate feasibility and expression ranges in a clinical population, patients with sarcoma were examined with a multi-agent imaging sequence of [^{11}C]-thymidine (Tdr) to quantify tumor proliferation, [^{18}F]-misonidazole (FMISO) to determine tumor hypoxic volume, [^{11}C]-verapamil (Verap) to assess P-glycoprotein activity, and [^{15}O]-water to quantify tumor blood flow (BF). Images were compared to FDG scans.
Materials and Methods: Ten patients with soft tissue sarcoma were imaged in this pilot study involving neo-adjuvant adriamycin chemotherapy. Studies with all imaging agents were done at baseline and prior to surgery. At mid-chemotherapy, FMISO and Tdr were repeated. Image analysis was by a five-compartment model for Tdr, a one-compartment model with BF normalization for Verap, and determination of tumor hypoxic volume in mL for FMISO. Patient outcome was measured as months from baseline scan to tumor recurrence, metastasis or death.
Results: Image analysis in this pilot study showed heterogeneity in tumor baseline levels for each agent and patient. These did not correlate with FDG uptake or show other associations, suggesting that expression for each of pathway is measuring an independent aspect of tumor biology. Repeat imaging during therapy showed that most patients had reductions in Tdr flux. Some patients showed return of FMISO hypoxia images to normoxic levels. For Verap there was a range of tumor uptake rates normalized to BF at baseline, but all patients showed a decreased Verap uptake in tumor after neo-adjuvant therapy but unchanged background muscle uptake. In this pilot dataset, high FMISO levels at baseline and decreased tumor Verap uptake after adriamycin were associated with the worst patient outcomes. This latter finding may indicate induction of P-gp multi-drug resistance and other genomic changes as a consequence of chronic hypoxia.
Conclusions: Multi-parameter PET imaging for assessment of tumor phenotype that may be predictive of poor outcome in sarcoma is feasible. The pathways imaged for each agent provided unique tumor measurements, which may predict risk for poor response. This pilot study in sarcoma patients supports further evaluation in a multi-center trial and similar protocols in multiple tumor histologies.
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651 POSTER DISCUSSION

Pharmacodynamic evaluation of pCDC2 and Wee1 signature as biomarkers of target engagement for the Wee1 tyrosine kinase inhibitor MK-1775

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Background: MK-1775 is a first-in-class inhibitor of Wee1, a kinase that phosphorylates CDC2 to inactivate the CDC2/cyclin B complex thereby regulating the G2/M checkpoint. Biomarkers that inform this class of therapeutics remain to be fully developed, including assays that demonstrate target engagement. Such biomarkers were evaluated in the context of a phase I first-in-man clinical trial of MK-1775.
Material and Methods: MK-1775 was administered p.o. in dose escalating cohorts both as monotherapy and in combination with either gemcitabine, cisplatin, or carboplatin. Pharmacokinetics (PK) and pharmacodynamics (PD) of MK-1775 were evaluated and benchmarked against targets identified in preclinical models. CDC2 phosphorylation (pCDC2) was assessed by immunohistochemistry (IHC) in serial skin biopsies obtained at baseline, 8 hrs or 48 hrs after MK-1775. Wee1 gene expression signature was analyzed by quantitative polymerase chain reaction (qPCR) from plucked hair samples.
Results: To date, 118 pts have been treated with MK-1775 monotherapy or in combination with chemotherapy at doses ranging from 25 mg to 1300 mg to define the maximum tolerated doses (MTD). Statistically significant, dose dependant decreases in pCDC2 were observed in skin biopsies from patients across multiple dose levels of MK-1775. Pooled analysis of pCDC2 by dose across chemotherapy arms in this study suggests that doses $\geqslant$100 mg MK-1775 appear to approach 50% inhibition of pCDC2. In contrast chemotherapy alone resulted in significant upregulation of pCDC2. Supporting evidence of target engagement was also observed with single agent MK-1775 modulation of a Wee1 gene expression signature.
PK increases were approximately dose proportional at all the tested dose levels of MK-1775 both as monotherapy and in combination with chemotherapy. A strong positive correlation between plasma MK-1775 concentrations and MK-1775 dose, and a negative correlation between plasma concentrations of MK-1775 and skin pCDC2 levels was seen.
Conclusions: MK-1775 is a first-in-class Wee1 inhibitor that demonstrates significant target engagement at tolerable doses both as a single agent and in combination with chemotherapy. Clinical activity was observed in combination with gemcitabine, cisplatin or carboplatin.

Chemoprevention

652 POSTER

The p53 pathway as a molecular target for the suppressive chemopreventive action of the histone deacetylase inhibitor tributyrin in rat hepatocarcinogenesis

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Epigenetic mechanisms and pathways involved with regulation of p53 nuclear-cytoplasm translocation have been proposed as molecular targets for carcinogenesis control. Histone deacetylase inhibitors (HDACi) such as tributyrin (TB), a butyric acid prodrug, represent promissing anti-cancer agents. In this study we evaluated the chemopreventive activity of TB when administered to rats during promotion phase of hepatocarcinogenesis. Moreover, epigenetic mechanisms and p53 pathway as molecular targets of TB were also investigated. After being submitted to the resistant hepatocyte model rats received TB (200 mg/100 g b.w.; TB group) or maltodextrin (300 mg/100 g b.w., isocaloric control; CO group) during 5 consecutive weeks. The macroscopic analysis of the livers revealed that compared to CO group, TB group presented smaller ($p < 0.05$) number of nodules. Hepatic GSTP-positive preneoplastic lesions (PNL) morphometry showed that compared to CO group, TB group presented smaller ($p < 0.05$) number, area and % of liver section occupied by persistent PNL (pPNL; sites of